10/5[redacted]8[redacted] FILE

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CONFIRMATION BY COURIER

10 June 2004

Dear Sirs,

European Patent Application No. PCT/GB03/0034
In the name of: NORCHIP A/S *et al*
Representative's Ref: SCB/P58858/001

I write in response to the Written Opinion dated 3rd May 2004 and enclose herewith amended claims 1 to 12 to replace the claims presently on file. The applicant is grateful to the Examining Authority for granting an extension of time for response.

For the avoidance of doubt, it is hereby stated that the Applicant reserves the right to reinstate any subject-matter removed by way of this amendment during prosecution in the National Phases. In addition, the Applicant reserves the right to file Divisional applications directed to any subject-matter removed by way of the amendment.

Summary of amendments

Amended claim 1 is based on former claim 6, but includes the feature that screening for E6 mRNA expression is carried out using isothermal amplification in combination with real-time detection of the amplification products. Basis for this claim is to be found on page 26, lines 29-32.

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Amended claim 2 is based on original claim 7, but again including the feature that screening for E6 mRNA expression is carried out using isothermal amplification in combination with real-time detection of the amplification products.

Dependent claim 3 specifies that the isothermal amplification is NASBA, transcription-mediated amplification, signal-mediated amplification of RNA or isothermal solution phase amplification. Basis for this claim is to be found on page 26, lines 25-28. Claim 4 specifies that E6 mRNA is detected by real-time NASBA. Basis for this feature is to be found in former claim 15.

Dependent claims 5 to 9 are based on original claims 8, 9, 11, 12 and 10 respectively.

New claim 10 relates to a kit for use in the detection of mRNA transcripts of the E6 gene(s) of HPV, the comprising one or more primer-pairs which enable amplification of a region of E6 transcripts from HPV types 16, 18, 31 and 33 by NASBA and one or more molecular beacon probes. Basis for this claim is to be found in the passage spanning page 29, line 20 to page 40, line 21, which contain extensive description of components to be included in kits, and particularly describes the features of NASBA primers and molecular beacon probes. Specific basis for inclusion of more primer-pairs which enable amplification of a region of E6 transcripts from HPV types 16, 18, 31 and 33 is to be found in the passage on page 30, lines 13-18. It is clear from the teaching of the application as a whole that HPV screening methods may be based on detection of E6 mRNA alone, as well as on detection of E6 and L1 in combination. Therefore, it would be clear to the skilled reader that kits which enable detection of E6 transcripts alone are contemplated. A preferred embodiment of such a kit is described in Example 5.

Claim 11 specifies that the kit contains separate primer-pairs specific for each of HPV types 16, 18, 31 and 33. Basis for this claim is to be found on page 30, lines 20-27.

Claim 12 relates to a kit comprising one or more primer pairs of defined sequence and accompanying identification probes. Basis for this claim is to be found in Example 5 of the application as originally filed.

Novelty

New independent claims 1 and 2 filed herewith are both novel over the prior art cited by the Examiner. None of the cited prior art documents disclose *in vitro* methods for screening to assess risk of developing cervical carcinoma or to identify subjects having abnormal cellular changes in the cervix based on detection of E6 mRNA expression using isothermal amplification in combination with real-time detection of the amplification products.

The disclosures of the prior art documents identified by the Examiner as being of relevance to assays based on detection of E6 mRNA expression are summarized below.

D4 describes a method for detecting and/or quantitating specific RNA transcripts of human papillomavirus. The method disclosed for detection of mRNA transcripts is based on hybridization of a nucleic acid probe to the mRNA. There is no disclosure of the use of an isothermal amplification reaction with real-time detection of the amplification products.

Documents D5 and D6 are both describe methods for detection of HPV mRNA based on reverse-transcription PCR, which is not an isothermal amplification technique. Detection of the amplification products appears to be based on the use of agarose gel electrophoresis-by analogy to the methods described for detection of HPV DNA. There is no disclosure of real-time detection of the amplification products and no disclosure of the use of an isothermal amplification technique.

Document D7 describes a 2-step nucleic acid hybridization probe assay for detection of mRNA from human papillomavirus. In this method E6 mRNA transcripts are first amplified using 3SR amplification. Once the amplification reaction is complete, the amplification products are detected. The detection reaction involves hybridization of the amplification products to a biotinylated reagent capture probe which enables attachment of the resulting complex to a solid phase. The immobilized capture complexes are then washed to remove bound and unreacted reagents and then hybridized with a reporter/conjugated detection probe, subjected to a second washing step and then detected using a suitable fluorogenic or chromogenic enzyme substrate. Accordingly, in this method the amplification reaction is completed before the products thereof are detected. This does not involve real-time detection of the products of an isothermal amplification reaction.

Document D8 describes the use of nucleic acid sequence-based amplification (NASBA) in the detection of E6/E7 transcripts of human papillomavirus. NASBA reaction products are visualized either by Northern Blot analysis using a HPV-specific radioactively labelled oligonucleotide probe or by a non-radioactive enzyme-linked gel assay (ELGA). Both of these detection techniques are carried out after the NASBA amplification reaction is complete. Thus, there is no disclosure in D8 of the use of real-time NASBA.

Document D9 describes a study of the splicing pattern of E6/E7 transcripts of HPV type 16 in various cell types. Splicing patterns of the E6/E7 transcripts are studied using RT-PCR, with the products of the PCR reaction being assessed by agarose gel electrophoresis. There is no disclosure of the use of an isothermal amplification reaction with real-time detection of the amplification products in order to assess E6/E7 mRNA expression.

D10 is concerned with a study of expression patterns of E6/E7 mRNA in biopsy tissue from different grades of cervical intraepithelial neoplasia. Detection of E6/E7 mRNA transcripts is carried out using RT-PCR, which is not an isothermal technique. Transcription products are quantitated by scintillation counting of radio labelled PCR products excised from a polyacrylamide gel. This technique does not constitute real-time detection of the amplification products.

Accordingly, the amended claims filed herewith must be acknowledged as novel over the cited prior art.

Inventive Step

There is no teaching or suggestion in any of the prior art documents cited by the Examiner of an HPV detection assay based on the use of isothermal amplification of mRNA transcripts in combination with real-time detection of the amplification products to detect E6/E7 expression.

D8 describes the application of the NASBA nucleic acid amplification technique in the detection of E6/E7 mRNA transcripts. As outlined above, in D8 the NASBA reaction products are visualized either by Northern Blot analysis using a HPV-specific radioactively labelled oligonucleotide probe or by a non-radioactive enzyme-linked gel assay (ELGA). Both of these detection techniques are carried out after the NASBA amplification reaction is complete. There is no teaching or suggestion that it is possible to detect the products of the NASBA reaction in real-time. The authors of D8 tested E6/E7-specific NASBA in comparison with PCR amplification of HPV DNA on nucleic acids extracted from fresh cervical smears. Two of the smears found positive for HPV type 16 DNA by PCR were negative in the NASBA reaction. The authors of D8 conclude that the discrepancy between the two methods may be due to a difference in sensitivity. Therefore, a skilled reader may conclude from this teaching that NASBA is less sensitive than detection techniques based on PCR amplification of HPV DNA. This may dissuade the skilled reader from trying to develop the NASBA technique as the basis of a diagnostic tool for HPV, where sensitivity, as well as specificity, is of extreme importance. In fact, the present inventors have shown that when used in combination with real-time detection, the NASBA technique may exhibit greater sensitivity than PCR amplification of HPV DNA, as illustrated in the examples of the present application (page 75, lines 35-40). There is nothing in D8 which would suggest to the skilled reader that real-time detection is even feasible in the context of HPV detection, let alone that this would improve sensitivity.

Document D7 describes a method for human papillomavirus detection which is based on RNA amplification followed by detection of the amplification products, the amplification being carried out by the 3SR reaction. On page 18 the authors of D7 explain that the assay format was devised in order to optimize the signal obtainable from specimens having low viral mRNA copy number. The inventors conclude that it is necessary to use fluid phase capture of the target RNA followed by immobilization onto a solid phase in order to achieve the required sensitivity. There is no suggestion that the required sensitivity can be achieved by detection of the RNA amplification products as they are formed, e.g. a using real-time approach. Thus the teaching of D7, which is concerned with a method of HPV detection based on RNA amplification, would also dissuade the skilled reader from attempting real-time detection of the products of the RNA amplification reaction.

Detection methods based on isothermal RNA-based amplification provide a significant advantage over PCR for detection of E6/E7 mRNA transcripts in the context of HPV detection. RT-PCR, including RT-PCR with real-time detection, can amplify double-stranded DNA, single-stranded DNA and mRNA. Therefore, RT-PCR can not distinguish between double-stranded viral genomic DNA and viral mRNA. In cervical samples, e.g. cervical smears, the amount of DNA and mRNA present is so large that it is practically impossible to remove all DNA from the sample before using RT-PCR. Therefore, when carrying out RT-PCR on such samples steps have to be taken to ensure amplification of mRNA and not DNA. In contrast, isothermal RNA-mediated techniques can not amplify double-stranded DNA, hence this problem does not arise. The inventors have now shown that it is possible to use real-time detection in combination with isothermal amplification in the context of HPV detection, and that this can result in an increase in sensitivity. This is not what the skilled reader would have expected based on the prior art, which tends to teach away from the use of real-time detection with isothermal amplification techniques in the context of HPV detection. Thus, the combination of isothermal amplification with real-time detection is particularly advantageous for detection of HPV transcripts, since it provides sensitivity, specificity and reliability whilst avoiding the problems inherent in RT-PCR based protocols.

In paragraph 2.4.1 of the Written Opinion headed "Final Remarks" the Examiner surmises that it is clear that the association between certain types of HPV, integration into the host cell genome, its influence in the malignant transformation process of cervical cells and the strong transcriptional activity of the E6/E7 transcription unit upon integration are well-known in the field since more than twelve years before the present priority date. In addition, the Examiner concludes that due to the clinical relevance of this finding, the topic has been the subject of intense research and a high number of documents have been published disclosing results based on this finding and the potential utility of E6/E7 mRNA as a marker in the diagnosis of cervical cancer.

The Examiner is correct in his view that a large number of studies have been published concerning the role of E6/E7 mRNA and in his opinion that the field of HPV detection is highly important both clinically and commercially, and thus highly competitive. Yet, despite the extensive amount of research in this area and the clinical and commercial importance of HPV diagnostics, at the priority date of the present application no-one had developed a diagnostic assay based on assessment of E6/E7 mRNA expression which exhibits sufficient sensitivity, specificity and reliability to be of clinical and commercial interest.

In fact, as far as the applicants are aware, they are the first to develop an assay method and kit for detection of E6 mRNA expression demonstrating real clinical potential. The inventor's approach is based on the use of an isothermal RNA-mediated amplification technique in combination with real-time detection of the amplification products in order to detect E6/E7 mRNA expression from major cancer-related HPV types. The inventor has found that the use of such an approach, for example real-time NASBA, provides substantial improvements in sensitivity, yet produces minimal false-negative results. The inventor's approach has been tested in extensive clinical studies and it has been demonstrated that the assay is selective for those patient samples containing persistent, transforming HPV infections, enabling identification those patients at high risk of developing carcinoma. The applicants are the first to clearly demonstrate the utility of an assay based on detection of E6/E7 mRNA expression alone in a clinical context.

As aforesaid, the applicants consider that their success in developing a sensitive, specific and reliable assay based on E6/E7 mRNA expression is due to the use of the novel combination of isothermal (RNA-mediated) amplification in combination with real-time detection of the amplification products to detect E6/E7 mRNA expression.

In summary, the inventor has developed a sensitive, specific and reliable assay for HPV based on detection of E6/E7 mRNA expression. This assay is specific for the persistent, transforming HPV infections that are associated with progression to carcinoma and therefore provides a direct indication of patient risk. The inventor's approach is based on the use of isothermal (RNA-mediated) amplification in combination with real-time detection of the amplification products. Such an approach is neither taught nor suggested by the cited prior art, and moreover provides substantial improvements over alternative molecular methods of HPV detection based on PCR amplification of HPV DNA. The clinical potential of the inventor's approach has been extensively demonstrated through studies on patient-derived samples (further unpublished clinical data is available). Therefore, the claims as presently worded, which reflect the use of isothermal (RNA-mediated) amplification in combination with real-time detection of the amplification products for detection of E6/E7 mRNA expression, must be acknowledged as both novel and inventive.

The Examiner's favourable reconsideration of this application is requested in view of the amendments and arguments presented herewith.

* Please acknowledge the safe receipt of this letter and its enclosures by endorsing and returning to me one of the enclosed copies of Form 1037.

Yours faithfully,

SETNA; Rohan Piloo
Authorized Representative

* Enclosures;

554207; NLW; SJW